

The role of TGF- β and APRIL in human milk IgA production and development of allergic disease in early childhood

To the Editor,

The neonatal period coincides with substantial development of the mucosal IgA responses. Naïve B cells in Peyer's patches, mesenteric lymph nodes, and mucosal lamina propria undergo class switch recombination (CSR) to an IgA₁ or IgA₂ in response to T-cell dependent (TD) or T-cell independent (TI) stimuli, including CD40L and IL-21, or BAFF, A Proliferation-Inducing Ligand (APRIL), and IL-10, respectively. TGF- β has an essential role in both TD and TI CSR. During the first weeks of life, TI stimulation has been suggested as the most likely mechanism by which the infant's naïve B cells undergo IgA CSR as initially TD response is slow.¹ However, reduced expression of APRIL and its receptors TACI and BCMA contribute to the paucity of IgA plasma cells in infant's gut until 1 month of age.² Some studies have shown that breastfed infants may also have a more rapid mucosal IgA production compared to formula-fed infants.³ However, little is known regarding the capacity of immune factors in human milk (HM) to boost IgA production.

The main objective was to measure HM levels of cytokines associated with IgA CSR and to assess their capacity to induce CSR and IgA production in naïve B cells isolated from umbilical cord blood by flow cytometry and RT-PCR for IgA₁ and IgA₂ excision circle transcripts (ECTs).

Utilizing ELISA, we detected APRIL, a previously not appreciated IgA CSR factor in HM with the highest concentrations, up to 1 μ g/ml, in colostrum and early milk (Figure 1A; $p < .0001$; $r^2 = .45$). The presence of APRIL was confirmed by Western blot (Figure 1B). To assess whether APRIL is involved in IgA CSR, we added recombinant APRIL to the B cell medium for 7 days. In CSR assay, this yielded multiple bands, indicating the presence of IgA₁ and IgA₂ ECTs, consistent with IgA CSR (Figure 1C). This was also seen with HM, indicating that physiologically relevant concentrations of APRIL in HM induce IgA CSR, although no IgA production was seen (Figure 1D). It

is noteworthy that BAFF, which is present in the medium and thus in our controls, did not induce IgA CSR.

TGF- β is a known IgA CSR factor and is present in HM; however, its capacity in HM to induce IgA production has not been shown before. Acid activation, required for TGF- β function, greatly increased the proportion of IgA₁ ($p = .004$) and IgA₂ ($p = .001$) expressing B cells, suggesting a role for HM TGF- β in IgA production (Figure 1E). Considering that APRIL does not require acid activation, we concluded that TGF- β , but not APRIL, is required for IgA production. Lastly, varying concentrations of APRIL and TGF- β in HM did not impact the amount of IgA production (Figure S1).

We also assessed the association of 25 HM cytokines, measured by Luminex at 3 months of age, with the development of food allergy or any atopy at 5 years of age in a birth cohort.⁴ Although the role of IgA is largely elusive in allergic diseases, induction of mucosal IgA responses in infancy may be a key factor in determining protection for dietary and environmental allergens, and may provide protection against allergic diseases.⁵ Levels of APRIL and IL-34 were lower and conversely, TGF- β_3 , IFN- λ_2 , sCD30, and IL-8 were higher in HM received by a child with a food allergy or any atopy by 5 years ($p < .05$ for each, Figure 2, Figure S2). IL-34 is a novel cytokine in HM, which shares the CSF-1R with M-CSF, previously identified as a marker of normal skin.⁶ A limitation of the cytokine data is that this assay was not validated for HM and hence results could be confounded by the presence of interfering substances such as soluble cytokine receptors.

We demonstrate for the first time the presence of APRIL in HM, capable of IgA CSR, and the capacity of TGF- β in HM to induce IgA production. Infants receiving HM with higher levels of APRIL had a lower incidence of allergic disease. Although this does not imply a causal relationship, the association could suggest a role for HM factors in protection against allergic diseases possibly through boosting infant IgA production.

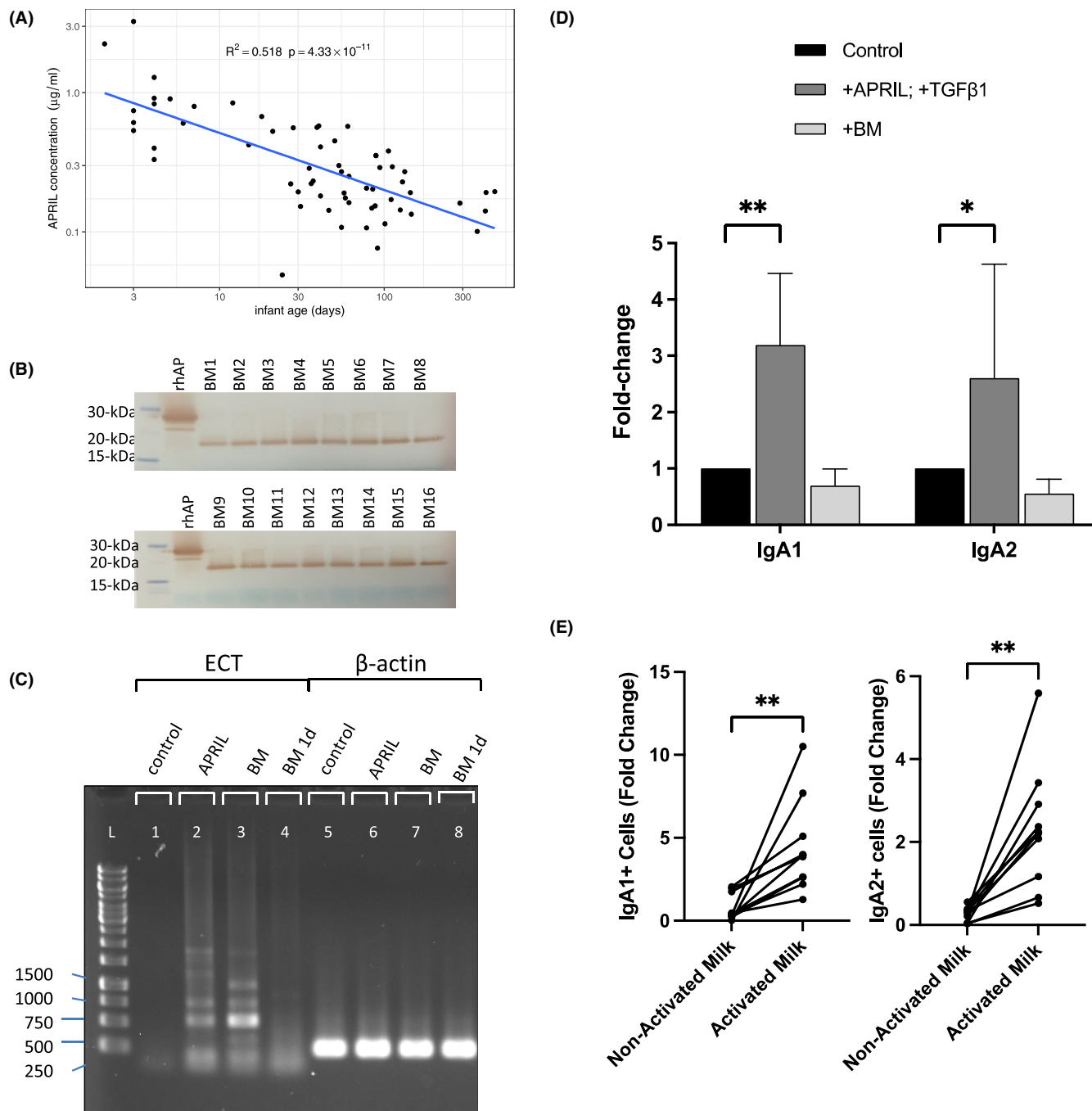


FIGURE 1 APRIL and TGF- β 1 or acid-activated milk induce IgA CSR in naïve B cells. (A) The concentration of APRIL in breast milk by ELISA (BM). (B) Western blotting using an anti-APRIL antibody confirmed the presence of APRIL in BM at ~18 kDa in 16 donors. Uncleaved, full-length recombinant APRIL (rhAP) was used as a positive control. (C) The addition of APRIL or BM to the B cell activation/proliferation medium for 7 days indicated the presence of IgA₁ and IgA₂ excision circle transcripts (ECTs, 550 and 650 bp, respectively). Media only (control) and BM treatment for 1 day (BM 1 day) did not show any bands. (D) Addition of biologically relevant concentrations of recombinant human APRIL and TGF- β 1 to the medium induced a significant increase in the proportion of IgA₁ and IgA₂ expressing B cells by flow cytometry compared to the medium alone (control). BM did not induce IgA. (E) Acid treatment to activate latent TGF- β in 10 BM samples induced a significant increase in IgA₁ and IgA₂ in cord blood IgD + B cells by flow cytometry. Fold-change was calculated relative to IgA production induced by a standard concentration of TGF- β 1 and APRIL. * $p < .05$, ** $p < .01$, *** $p < .001$

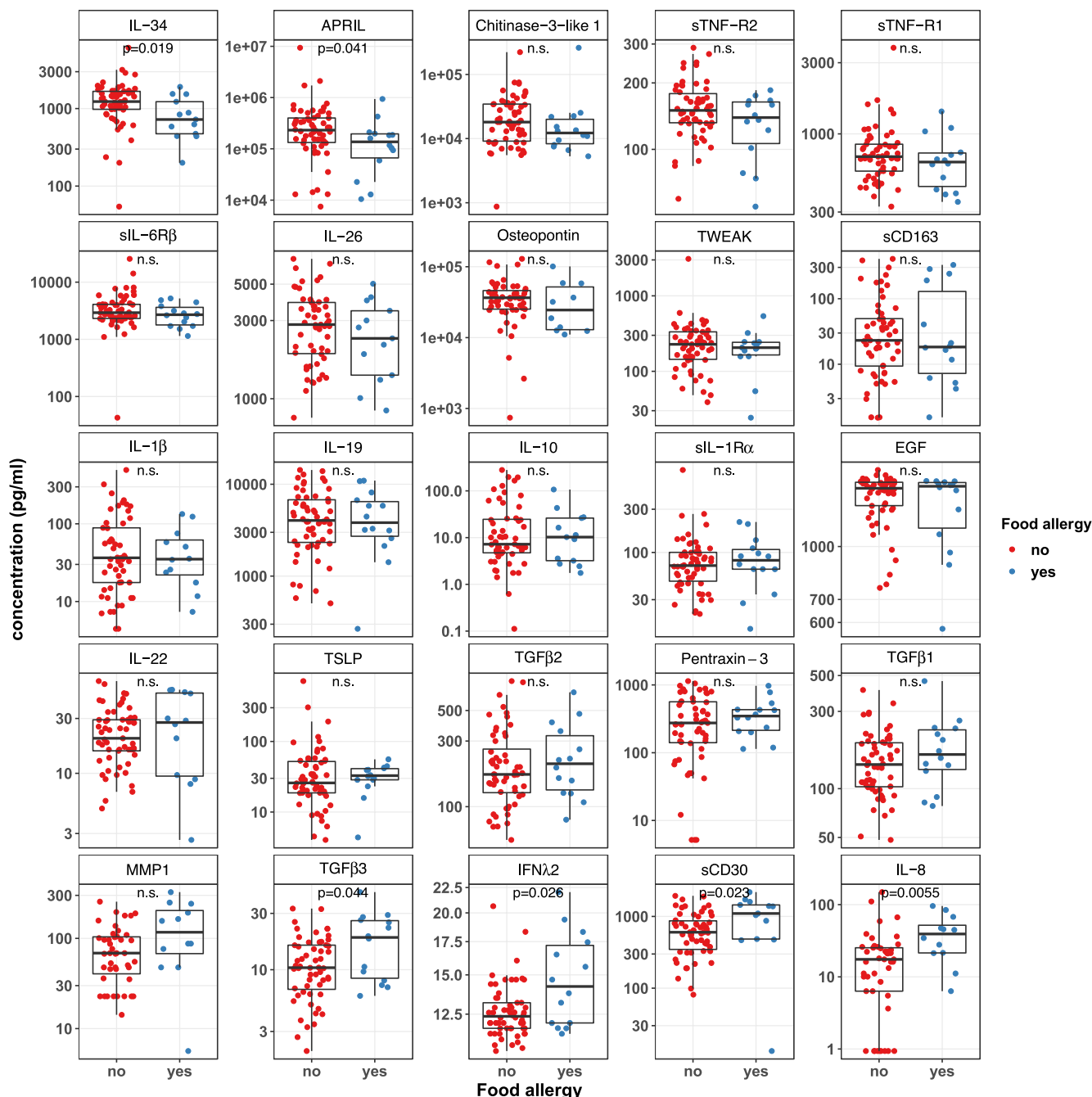


FIGURE 2 Cytokine concentrations as a function of infant food allergy status at 5 years of age. Cytokine concentrations were measured from FLORA birth cohort HM samples, collected at 3 months of lactation, using Luminex bead arrays. Infants were followed for 5 years and their food allergy status (yes vs no; the latter not excluding other atopic diseases or sensitization) was assessed as described in Supplementary Methods. HM cytokine concentrations are compared between mothers with a child developing food allergy (blue dots) or not (red dots). *p*-values are calculated using a *t*-test with log-transformed cytokine concentrations. The values are exploratory only, not corrected for multiple testing, and have to be verified in a larger cohort

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AUTHOR CONTRIBUTIONS

Antti Seppo: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (lead); Project administration (equal); Supervision (equal); Validation (equal); Visualization (lead); Writing—original draft (equal); Writing—review & editing (equal). **Puja Sood Rajani:** Formal analysis (equal); Investigation (equal); Methodology (equal); Writing—original draft (equal). **Alanna Wong:** Formal analysis (equal); Investigation (equal); Methodology (equal); Writing—original draft (equal). **John Varrone:** Formal analysis (equal); Investigation (equal); Methodology (equal). **Sade Fridy:** Investigation (equal); Methodology (equal). **Mikael Kuitunen:** Conceptualization (equal); Resources (equal). **Kaarina Kukkonen:** Conceptualization (equal); Resources (equal). **Erkki Savilahti:** Conceptualization (equal); Resources (equal). **Kirsi M. Jarvinen:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Project administration (equal); Resources (equal); Supervision (equal); Validation (equal); Writing—original draft (equal); Writing—review & editing (equal).

CONFLICT OF INTEREST

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Antti E. Seppo¹ 
 Puja S. Rajani¹
 Alanna Wong¹
 John J. Varrone¹
 Sade T. Fridy¹
 Mikael Kuitunen²
 Kaarina Kukkonen²
 Erkki Savilahti²
 Kirsi M. Järvinen¹ 

¹Department of Pediatrics, Division of Pediatric Allergy and Immunology, University of Rochester Medical Center, Rochester, New York, USA

Email: Kirsi_Jarvinen-Seppo@urmc.rochester.edu

²New Children's Hospital, Helsinki University Hospital and University of Helsinki, Helsinki, Finland

Correspondence

Antti E. Seppo, Department of Pediatrics, Division of Pediatric Allergy and Immunology, University of Rochester Medical Center, 601 Elmwood Ave., Rochester, NY 14642, USA.

Email: Antti_Seppo@urmc.rochester.edu

Editor: Alexandra Santos

ORCID

Antti E. Seppo  <https://orcid.org/0000-0001-5757-5268>

Kirsi M. Järvinen  <https://orcid.org/0000-0003-1548-6712>

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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